

# 篇蓄乙酸乙酯部位化学成分的抑菌活性研究

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**摘要:**篇蓄(*Polygonum aviculare* L.)是一种常用中药,具有杀虫止痒,治疗黄疸等功效。本文采用打孔法分别对其乙酸乙酯部位分离得到的十个化合物进行了抑菌活性实验,结果表明,除化合物**1**、**2**、**9**、**10**外,其它化合物均对不同的菌种呈现出不同的抑菌活性,并且呈一定的剂量效应。本实验为篇蓄及其化学成分作为天然的抑菌活性物质研究开发提供理论依据。

**关键词:**篇蓄;抑菌活性;Myricetin-3-*O*-(3'-*O*-galloyl)-rhamnopyranoside;抑菌圈

中图分类号:R284.1

文献标识码:A

DOI:10.16333/j.1001-6880.2016.4.014

## Antibacterial Activities of Compounds from Ethyl Acetate Extract of *Polygonum aviculare* L.

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**Abstract:** *Polygonum aviculare* L. was used for the treatment of cutaneous pruritus and jaundice. In current study, antibacterial activities of ten compounds isolated from ethyl acetate extract of *P. aviculare* were tested. The results showed that these compounds (except for **1**, **2**, **9** and **10**) showed inhibitory activity against different bacteria with dose-dependent effect. This indicated that *P. aviculare* and its components can be regarded as a potential source of natural antibacterial agent in the prevention and treatment of microbial infections.

**Key words:** *Polygonum aviculare* L.; antibacterial activity; myricetin-3-*O*-(3'-*O*-galloyl)-rhamnopyranoside; inhibition zone

## Introduction

*Polygonum aviculare* L., belonging to the family of *Polygonaceae*, is an annual or perennial prostrate herbaceous plant with small elliptic lanceolate leaves, and was widely distributed in the world<sup>[1]</sup>. *P. aviculare* contains a variety of chemical active constituents, such as flavonoids, phenylpropanoids, phenolic acids, amino acids and carbohydrates (flavonoids are the main chemical compositions)<sup>[2,3]</sup>. Some studies proved that *P. aviculare* had many functions and was used as diu-

retic, insecticide detoxification and antioxidant, etc<sup>[4-7]</sup>. We had reported the antibacterial activities and constituents of ethyl acetate extract of *P. aviculare* in the published paper. The ethyl acetate extract of *P. aviculare* (EAE) had obvious antibacterial activities for *Escherichia coli*, enteropathogenic *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Shigella dysenteriae*<sup>[8]</sup>. In this study, the main work was evaluating the antibacterial activities *in vitro* of ten compounds purified from EAE. There were methyl gallate (**1**), stigmast-5-en-3-*O*- $\beta$ -*D*-glucopyranoside (daucosterol, **2**), kaempferol (**3**), quercetin (**4**), gallic acid (**5**), arabinofuranoside (avicularin, **6**), quercetin-3-*O*- $\alpha$ -*L*-rhamnopyranoside (quercitrin, **7**), myricetin-3-*O*- $\alpha$ -*L*-(3'-*O*-galloyl)-rhamnopyranoside (**8**), 3, 3', 4', 5, 5', 7-

Received: October 13, 2015 Accepted: January 12, 2016

Foundation item: Talent Fund of Anhui agricultural university (yi2010-03)

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hexahydroxyflavone-3-*O*- $\alpha$ -*L*-rhamnopyranoside (myricitrin, **9**) and kaempferol-3-*O*- $\alpha$ -*L*-rhamnopyranoside (juglanin, **10**). The results of this study provided theoretical foundation for the utilization of *P. aviculare* in the health needs.

## Materials and Methods

### Samples

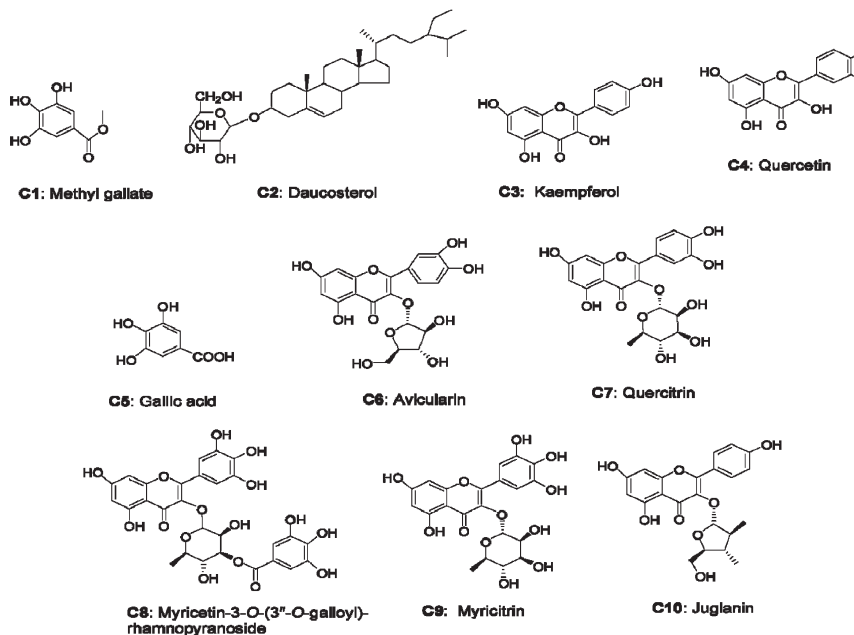


Fig. 1 Chemical structures of compounds from ethyl acetate extract of *P. aviculare*

### Reagents

Berberine hydrochloride was purchased from Nanjing Baijingyu Pharmaceutical Co., Ltd. China and norfloxacin was purchased from Anhui Sanjinwansen Pharmaceutical Co., Ltd. China. All other chemicals and reagents used in this study were of analytical grade and supplied by Tianjin Bodi Chemicals Co., Ltd. (Tianjin, China).

### Microorganisms

The tested microorganisms included *Escherichia coli*, enteropathogenic *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Shigella dysenteriae*. All of them were obtained from Key Laboratory of Tea Biochemistry & Biotechnology (LTBB), Anhui Agricultural University. All bacterial strains were respectively cultivated in fluid nutrient medium (Beef extract, 5 g/L; Peptone, 10 g/L; NaCl 5 g/L; pH 7.4), and incubated

Ten compounds were isolated from ethyl acetate extract of *P. aviculare* (Fig. 1). The separation and identified of the compounds were described in the published paper<sup>[8]</sup>. The plant materials were gathered from Dabie Mountain (in Jinzhai County, Anhui Province, PR China. in September 2009 and was identified by Prof. Shoujin Liu in School of Pharmacy, Anhui University of Traditional Chinese Medicine.

at 37 °C for 24 h. After incubated, 1 mL bacterial suspension was added in the 100 mL new fluid nutrient medium, and incubated at 37 °C for 12 h. The new bacterial suspension was ready for further processing.

### Antibacterial assays

The hole plate diffusion method was used to investigate the antimicrobial activities of the purified compounds. They were diluted by DMSO to different gradient concentrations (200  $\mu$ g/mL, 400  $\mu$ g/mL and 800  $\mu$ g/mL for each compound). Berberine hydrochloride (25.6 mg/mL) and norfloxacin (25  $\mu$ g/mL) diluted by DMSO as standard antimicrobials were used for comparison.

For the determination of antibacterial activity, 300  $\mu$ L bacterial suspension were inoculated onto 15 cm diameter plates with 45 mL nutrient agar medium (Beef extract, 5 g/L; Peptone, 10 g/L; NaCl 5 g/L; Agar, 20 g;

pH 7.4). 50  $\mu$ L of testing solution was injected in the hole of 6 mm size prepared using sterile steel tuber. These plates were incubated at 37  $^{\circ}$ C for 24 h. Antimicrobial activity of test solution was determined by measurement of inhibition zone against the reagent (DMSO) blank. All the tests were performed in triplicate.

### Data analysis

Data were analyzed by SPSS (Version 11.0 for Windows, SPSS Inc., Chicago, IL) and expressed as mean  $\pm$  SD of triplicate determinations.

### Results and Discussion

Table 1 illustrated the antibacterial activities of ten

**Table 1 The inhibition zone of compounds for testing microorganisms**

	Concentration ( $\mu$ g/mL)	Diameter of inhibition zone (mm)				
		<i>Escherichia coli</i>	Enteropathogenic <i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella typhi</i>	<i>Shigella dysenteriae</i>
DMSO	–	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
Berberine Hydrochloride	25.6 $\times$ 10 <sup>3</sup>	10.1 $\pm$ 0.1 * *	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
Norflloxacin	25	24.2 $\pm$ 0.1 * *	32.2 $\pm$ 0.1 * *	33.2 $\pm$ 0.2 * *	32.2 $\pm$ 0.3	33.9 $\pm$ 0.1 * *
Methyl gallate	200	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	400	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	800	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
Daucosterol	200	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	400	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	800	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
Kaempferol	200	9.9 $\pm$ 0.1 * *	6.0 $\pm$ 0.0	10.1 $\pm$ 0.1 * *	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	400	10.1 $\pm$ 0.1 * *	11.9 $\pm$ 0.2 * *	15.1 $\pm$ 0.1 * *	6.0 $\pm$ 0.0	9.9 $\pm$ 0.2 * *
	800	11.1 $\pm$ 0.1 * *	12.1 $\pm$ 0.1 * *	13.0 $\pm$ 0.1 * *	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
Quercetin	200	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	400	6.0 $\pm$ 0.0	8.2 $\pm$ 0.2 * *	10.1 $\pm$ 0.1 * *	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	800	6.0 $\pm$ 0.0	10.2 $\pm$ 0.1 * *	11.1 $\pm$ 0.1 * *	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
Gallic acid	200	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	400	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	800	6.0 $\pm$ 0.0	9.3 $\pm$ 0.2 * *	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
Avicularin	200	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	400	6.0 $\pm$ 0.0	10.94 $\pm$ 0.1 * *	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	800	6.0 $\pm$ 0.0	11.1 $\pm$ 0.1 * *	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
Quercitrin	200	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	10.1 $\pm$ 0.1 * *	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	400	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	13.1 $\pm$ 0.2 * *	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	800	6.0 $\pm$ 0.0	12.1 $\pm$ 0.1 * *	15.1 $\pm$ 0.2 * *	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
Myricetin-3-O-(3''-O-galloyl)- rhamnopyranoside	200	6.0 $\pm$ 0.0	12.2 $\pm$ 0.2 * *	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	400	6.0 $\pm$ 0.0	10.1 $\pm$ 0.1 * *	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	800	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
Myricitrin	200	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	400	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	800	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
Juglanin	200	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	400	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	800	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0

Note: The diameter of inhibition zone > 6.0 mm indicated that the substance had antibacterial activity, \* \*  $P < 0.01$  means significantly different Vs blank Control (DMSO).

compounds. Methyl gallate, daucosterol, myricitrin and juglanin did not show any antibacterial activity in this study. The other compounds showed different antibacterial activities and displayed significantly different Vs blank Control ( \*\*  $P < 0.01$  ).

Kaempferol can restrain the growth of *Escherichia coli* ( at 200, 400  $\mu\text{g/mL}$  and 800  $\mu\text{g/mL}$  ), enteropathogenic *Escherichia coli* ( at 400  $\mu\text{g/mL}$  and 800  $\mu\text{g/mL}$  ), *Staphylococcus aureus* ( at 200, 400  $\mu\text{g/mL}$  and 800  $\mu\text{g/mL}$  ) and *Shigella dysenteriae* ( at 400  $\mu\text{g/mL}$  ). But it did not have any effect for *Salmonella typhi*. Quercetin appeared its antibacterial activity merely at 400  $\mu\text{g/mL}$  and 800  $\mu\text{g/mL}$  against enteropathogenic *Escherichia coli* and *Staphylococcus aureus*. Gallic acid (800  $\mu\text{g/mL}$ ) and avicularin (400  $\mu\text{g/mL}$  and 800  $\mu\text{g/mL}$ ) had antibacterial activities only for enteropathogenic *Escherichia coli* and had hardly any effect for other bacterium. Quercitrin (200  $\mu\text{g/mL}$ , 400  $\mu\text{g/mL}$  and 800  $\mu\text{g/mL}$ ) showed good suppression effect for *Staphylococcus aureus*, and it merely inhibited the growth of enteropathogenic *Escherichia coli* at 800  $\mu\text{g/mL}$ . Myricetin-3-*O*-(3''-*O*-galloyl)-rhamnopyranoside was not showing any inhibition for all bacteria except enteropathogenic *Escherichia coli*. The inhibition zone of myricetin-3-*O*-(3''-*O*-galloyl)-rhamnopyranoside against enteropathogenic *Escherichia coli* was 12.2 mm (200  $\mu\text{g/mL}$ ) and 10.1 mm (400  $\mu\text{g/mL}$ ). But the activity was not obvious at 800  $\mu\text{g/mL}$ .

The best inhibition performance for *Escherichia coli*, enteropathogenic *Escherichia coli* and *Staphylococcus aureus*, were kaempferol at 800  $\mu\text{g/mL}$ . Myricetin-3-*O*-(3''-*O*-galloyl)-rhamnopyranoside can inhibit the growth of Enteropathogenic *Escherichia coli* 12.2 mm ( the diameter of inhibition zone ) at 200  $\mu\text{g/mL}$  while kaempferol 12.1 mm at 800  $\mu\text{g/mL}$ . Quercitrin can inhibit the growth of *Staphylococcus aureus* 15.1 mm as kaempferol, but its concentration (800  $\mu\text{g/mL}$ ) was higher than kaempferol (400  $\mu\text{g/mL}$ ). These com-

pounds cannot suppress the growth of *Salmonella typhi* in our experiments. Through the survey of antibacterial activities of these compounds, the roles of them in antibacterial test *in vitro* were confirmed. This study had improved the possibility of the use of *P. aviculare* in antimicrobial drug and food additive development for human application.

### Acknowledgement

The authors are grateful to the natural product team of Key Laboratory of Tea Biochemistry & Biotechnology (LTBB) providing the generous help. We also thank the Anhui Agricultural University for financial support. We all thank Professor Guanhu Bao for providing the help of article amending.

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